Design and Synthesis of some novel 3-benzylidene-1*H*-benzo [4,5]imidazo[2,1-c] [1,4]oxazine-1,4(3*H*)-dione derivatives and evaluation of their biological activity

Sunitha Ch. and Brahmeshwari G.*
Department of Chemistry, Kakatiya University, Warangal-506009, INDIA
*sunitha.c82@gmail.com

Abstract

A novel series of 3-arylbenzylidene-1H-benzo[4,5] imidazo[2,1-c][1,4]oxazine-1,4(3H)-dione (4a-l) was synthesized in moderate to good yields. The structures of the newly synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR mass spectrometry and elemental analysis. The synthesized compounds were evaluated for their anti-bacterial and anti-oxidant activities. Among all the derivatives tested, the compound 3-(3,5-dimethoxy benzylidene)-1H-benzo [4,5]imidazo[2,1-c][1,4]oxazine-1,4-(3H)-dione showed more potent activity and 3-(4-methoxybenzylidene)-1H benzo[4,5] imidazo[2,1-c][1,4]oxazine-1,4(3H)-dion exhibited good activity as compared to standard streptomycin.

The anti-fungal activity reveals that the compound 3-(3,5-dimethoxy benzylidene)-1H-benzo[4,5]imidazo [2,1-c][1,4]oxazine-1,4-(3H)-dione showed more promising activity as compared to standard Itrazole. The remaining compounds showed moderate to poor activity. Similarly, the anti-oxidant activity results revealed that the compound 3-(3,5-dimethoxy benzylidene)-1H-benzo [4,5] imidazo[2,1-c][1,4] oxazine-1,4-(3H)-dione exhibited potent activity as compared to the other tested compounds.

Keywords: Benzimidazole fused oxazine-1,4-dione, *in vitro* anti-bacterial and anti-oxidant activity.

Introduction

Nowadays the infection diseases are caused by different types of bacteria which are pathogenic. Bacteria are microscopic unicellular organism and most of them are beneficial to human and some of them are harmful which are growing threats to human health during the past few decades. The decrease in sensibility to antimicrobial agents has also been increasing variety of pathogens. The inhibitory properties as regard to representative fungi have been exploited. However, pathogenic bacteria develop resistance against countless antimicrobial agents.

Especially benzimidazole is promising moiety to design novel scaffolds with antibacterial activity[1-4] and important classes of active substances in the field of medicine and pesticides having a wide range of biological activities such as potent antifungal[5], antimicrobial[6], anti-hypertensive[7], anti-HIV[8], antioxidant and antitumor agents[9,10] anti-cancer[11,12] anti-allergic[13], anti-HIV[14,15] anti-tubercular[16,17] and anti-inflammatory agents[18]. This benzimidazole core is a privileged sub-structure and is present in a number of bio-molecules including DNA purine bases and vitamin B_{12} as well as in a range of therapeutic agents[19]. In present situation, there is urgent need for the development of novel benzimidazole derivatives with promising anti-microbial activity. Based on the facts, here we describe the synthesis of novel benzimidazole derivatives and screened for their anti-microbial and anti-oxidant activity.

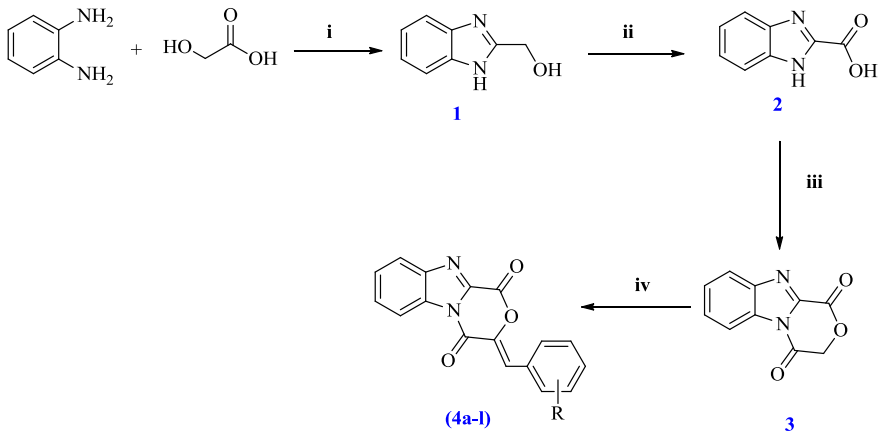
Material and Methods

Melting points were determined in open capillary and are uncorrected. All the chemicals and solvents used were purchased from Sigma-Aldrich. Column chromatography was performed using silica gel (60-120 mesh size) purchased from Sigma-Aldrich and thin Layer chromatography was carried out using aluminium sheet pre-coated with silica gel $60F_{254}$ purchased from Merck. ^{13}C NMR spectra were run on Jeol spectrometer (75 MHz) using TMS as internal standard and DMSO-d_6 as a solvent.^{1}H NMR data were recorded in DMSO on a Bruker Avance 400MHz spectrometer. The mass spectra were measured on a Jeol SX-102 spectrometer. CHN analysis was carried out on a Carlo Erba EA 1108 automatic analyzer. Combustion analyzer was found to be within the limits of permissible limits.

4a: R=C_6H_5, **4b:** R= 4-$CH_3C_6H_4$, **4c:** R= 2,4-dimethyl C_6H_3, **4d:** R= 4-$NO_2C_6H_4$, **4e**: R= 4-ChloroC_6H_4, **4f:** R= 3,5-ClC_6H_3, **4g:** R= 4-BrC_6H_4, **4h:** R= 4-$OCH_3C_6H_4$, **4i:** R= 3,5-$OCH_3C_6H_3$, **4j:** R= 3,4-ClC_6H_3, **4k**: R=4-CNC_6H_4, **4l**: R=4-FC_6H_4.

Reagents and Conditions: (i) 4N HCl, reflux, 4h, 90^0C, (ii) 20%NaOH, $KMnO_4$, reflux, 1h. (iii) dry THF, Cs_2CO_3, Chloro acetyl chloride, reflux, 80^0C, 5-6h and (iv) EtOH, R-Ar-CHO, *p*-TsCl, reflux, 3h.

***In vitro* Anti-bacterial activity:** The target compounds (4a-l) were screened for their *in vitro* antibacterial activity against gram-positive and gram-negative bacterial strains by the broth dilution method[21] by using streptomycin as positive control. The results are summarized in table 1. The test compounds and standard drugs are dissolved in DMSO of specific concentrations 200 and 400µg/mL. Among the tested compounds, 3-(3,5-dimethoxybenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4-(3*H*)-dione (4i) showed excellent inhibition against all the tested bacterial strains with ZOI ranging from 9.43 to 27.89 mm (200 and 400 µg/mL) respectively.



Scheme 1

Similarly, the compound 3-(4-methoxybenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4(3*H*)-dione (4h) shows promising activity with range of inhibition activity from 11.25 to 26.22 mm (200 and 400 µg/mL).

Compounds 3-(3,5-dichlorobenzylidene)-1*H*-benzo[4,5] imidazo[2,1-*c*][1,4] oxazine-1,4(3*H*)-dione (4f) and 3-(3,4-dichlorobenzylidene)-1*H*-benzo[4,5]imidazo [2,1-*c*][1,4] oxazine-1,4-(3*H*)-dione (4j) exhibited moderate activity ranging from 17.45 to 33.15 mm (200 and 400 µg/mL).These results reveal that the electron withdrawing groups of phenyl decrease the activity compared to electron donating substituent on phenyl nucleus, while the rest of the compounds have shown poor activity against all the tested bacterial strains when compared to standard drug Streptomycin.

Anti-fungal activity against *Candida albucance:* *Fusariumoxysporium* using itrazole as positive control and results is summarized in table 1. The compounds (4i) and (4h) bearing methoxy groups as a substituent on phenyl nucleus show more potent activity against all tested fungal strains as compared to standard drug. Moreover, the compound (4f) exhibited good activity due to the presence of electron withdrawing group whereas the remaining compounds exhibited poor to moderate activity compared standard drug.

Anti-oxidant activity of compound (4a-l): All the synthesized compounds (4a-l) were screened for free radical scavenging activity in terms of hydrogen donating or radical scavenging ability by 2, 2-diphenyl-1- picryl hydrazyl (DPPH) method.[22] Methanol (95%), DPPH solution and standard compound (ascorbic acid) were used as blank, control and reference respectively and results are tabulated in table 2.

The examination of free radical scavenging ability of the synthesized compounds (4a-l) results showed that compounds (4f), (4i) and (4h) have exhibited excellent antioxidant activity with IC_{50} values ranging from 6.24±0.42 to 15.02±0.22 µM. Remaining compounds have shown moderate to weak scavenging ability with IC_{50} values ranging from 16.29±0.51 to 34.62±0.42. It is to point out that all the potent analogues which contain electron withdrawing substituent like bromo, chloro, nitro and cyano groups on the benzene ring showed moderate activity and electron donating substituent exhibited more potent activity compared to standard compound.

Synthesis of (1*H*-benzo[d]imidazol-2-yl) methanol (1): To a round bottom flask containing *o*-phenylenediamine (100 mmol) in 100 mL of a solution of hydrochloric acid (4N), add 3 equivalents of glycolic acid (100 mmol) reflux and the reaction mixture for 4 h at 90°C. The progress of the reaction is monitored by TLC. After completion of the reaction, the reaction mixture is cooled at room temperature, then the solution pH is adjusted to 8 using NH_4OH (10%). The precipitate was filtered and dried to afford 1 as a white solid.

Synthesis of 1*H*-Benzo[d]imidazole-2-carboxylic acid (2): To a mixture of 1*H*-benzo[d]imidazol-2-yl) methanol (1) (20 mmol) and 20% NaOH (10 mL) add 20 mL of KMnO4 (5 mmol) in batchwise, the reaction mixture was refluxed for 1h. The progress of the reaction is monitored by TLC. After completion of the reaction, the reaction mixture is cooled at room temperature. Now the reaction mixture was diluted with water and filtered. The pH of the filtrate was adjusted to 5 with 6N HCl at 0°C. Filter the precipitate and dry to obtain 1*H*-Benzo[d]imidazole-2-carboxylic acid (2) as a white solid.

Synthesis of 1*H*-benzo[4,5]imidazo[2,1-*c*]oxazine-1,4-(3*H*)-dione (3): To a mixture of 1*H*-benzo[*d*]imidazole-2-carboxylic acid (2) (20 mmol) in dry THF (10 mL), add dried Cs_2CO_3 (40 mmol); slowly chloro acetyl chloride (20 mmol) was added, the mixture was stirred at 80°C for 5-6 h.

After completion of the reaction, the reaction mixture was concentrated in *vacuum* and re-dissolved in water (20 mL). The resulting solution was acidified to pH 6 with 6N HCl at 0°C and then extracted with ethyl acetate, the organic layers was combined and washed with saturated NaCl and dried over anhydrous sodium sulphate, filtered and concentrated under *vacuum* to give a white solid.

Table 1
***In vitro* anti-bacterial activity of compounds (4a-l) (µg /mL).**

Compounds	Ar	µg/mL	Zone of inhibition (*in mm*)					
			E. Coli	***Pseudomonas. aeruginoa***	***Staphylo coccus. aureus***	***Streptococcus. pneumoniae***	***Candida albucance***	***Fusariumoxy sporium***
(4a)	-C_6H_5	200	35.34	42.45	37.12	42.22	43.23	NA
		400	NA	34.20	42.29	33.50	38.23	42.10
(4b)	4-$CH_3C_6H_4$	200	25.23	NA	45.56	43.92	39.45	40.23
		400	34.52	42.17	36.61	41.23	37.34	36.56
(4c)	2,4-dimethyl C_6H_3	200	NA	NA	34.15	46.34	39.45	41.34
		400	45.50	NA	36.23	41.23	45.56	NA
(4d)	4-$NO_2C_6H_4$	200	36.23	35.32	42.32	41.02	37.23	41.36
		400	34.10	37.23	38.12	43.00	NA	38.45
(4e)	4-ChloroC_6H_4	200	NA	NA	NA	42.16	42.43	NA
		400	36.18	NA	34.14	35.15	NA	39.12
(4f)	3,5-ClC_6H_3	200	16.34	**18.21**	**17.45**	**29.65**	**19.34**	**23.45**
		400	18.56	**24.22**	**27.56**	**30.65**	**24.67**	**32.23**
(4g)	4-BrC_6H_4	200	35.36	35.52	36.10	38.01	NA	36.34
		400	46.85	47.22	34.56	45.29	36.13	41.23
(4h)	4-$OCH_3C_6H_4$	200	11.25	**16.36**	**14.34**	**26.13**	**17.89**	**21.04**
		400	13.45	**25.12**	**25.21**	**26.22**	**21.34**	**31.23**
(4i)	3,5-$OCH_3C_6H_3$	200	9.43	**14.29**	**10.23**	**12.14**	**15.65**	**17.83**
		400	10.02	**21.23**	**21.23**	**23.12**	**19.05**	**27.89**
(4j)	3,4-ClC_6H_3	200	19.23	**20.36**	**18.26**	**31.08**	**23.56**	**25.45**
		400	23.01	**26.04**	**29.12**	**33.15**	**27.43**	**35.67**
(4k)	4-CNC_6H_4	200	35.16	32.21	34.02	36.00	34.19	42.36
		400	40.06	40.10	43.08	41.66	36.45	38.12
(4l)	4-FC_6H_4	200	32.34	33.02	35.15	36.76	38.34	39.45
		400	41.26	56.26	45.66	45.68	38.12	41.34
Streptomycin		200	6.34	12.21	8.54	9.23	39.45	38.21
		400	8.45	19.02	16.45	19.10	37.45	36.34
Itrazole		200	-	-	-	-	12.32	16.45
		400	-	-	-	-	17.23	25.53

Table 2
Anti-oxidant activity of compounds(4a-l) (µM).

Compound	Ar	IC_{50} in µM[b]
(4a)	-C_6H_5	26.34±0.33
(4b)	4-$CH_3C_6H_4$	23.10±0.21
(4c)	2,4-dimethyl C_6H_3	31.16±0.20
(4d)	4-$NO_2C_6H_4$	34.62±0.42
(4e)	4-ChloroC_6H_4	32.52±0.53
(4f)	3,5-ClC_6H_3	**15.02±0.22**
(4g)	4-BrC_6H_4	31.55±0.26
(4h)	4-$OCH_3C_6H_4$	**9.19±0.34**
(4i)	3,5-$OCH_3C_6H_3$	**6.24±0.42**
(4j)	3,4-ClC_6H_3	16.29±0.51
(4k)	4-CNC_6H_4	29.12±0.78
(4l)	4-FC_6H_4	32.22±0.18
Ascorbic acid		3.78±0.43

Color: white solid, Yield. 72%. m.p:225-227°C; ^{1}H NMR (400 MHz, DMSO-*d6*, δ ppm): 5.24 (s,2H,-CH_2), 7.45 (t,1H, *J*=4.4Hz, benz-H), 7.53 (t,1H, *J*=4.7Hz, benz-H), 7.70 (d,1H, *J*=5.2Hz, benz-H), 7.96 (d,1H, *J*=5.2Hz, benz-H).

General procedure for the synthesis of 3-benzylidene-1*H*-benzo[4,5]imidazo[2,1-*c*] [1,4] oxazine-1,4(3*H*)-dione (4 a-l): To a mixture of 1*H*-benzo[4,5]imidazo[2,1-*c*]oxazine-1,4-(3*H*)-dione (3) (10 mmol) dissolved in EtOH (10 mL), add substituted benzaldehyde (10 mmol) and a catalytic volume of *p*-toluene sulphonyl chloride (*p*-TsCl) (0.5 mL) slowly. Then, the reaction mixture was slowly heated and refluxed for 3 hours. After heating the reaction mixture, cool to room temperature and filter, wash with water and dry in vacuum to get the crude product. The crude product was purified by column chromatography using 2:8 ethyl acetate and hexane as gradient to yield well to moderate yields.

3-benzylidene-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4(3*H*)-dione(4a): Color: Pale white solid, Yield. 67%. m.p:256-258°C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): δ 6.98 (s, 1H, =CH), 7.42 (d, 2H, Ar-H), 7.46 (d, 2H, *J*=4.3Hz, Ar-H), 7.50 (t, 1H, *J*=4.3Hz, Ar-H), 7.53 (t, 1H, *J*=3.6Hz, benz-H), 7.59 (t, 1H, *J*=3.8Hz, benz-H), 7. 66 (d, 1H, *J*=4.7Hz, benz-H), 7. 73 (d, 1H, *J*=4.8 Hz, benz-H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 157.2, 154.1, 147.1, 143.0, 136.7, 134.6, 132.3, 131.5, 128.2, 127.2, 125.2, 123.1, 122.4, 118.2, 115.4; MS: *m*/*z* 291 (M+H); Anal. Cacld for $C_{17}H_{10}N_2O_3$: C, 70.34; H, 3.47; N, 9.65. Found: C, 70.33; H, 3.46; N, 9.94 %.

3-(4-methylbenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*] [1,4]oxazine-1,4(3*H*)-dione(4b): Color: Pale white solid, Yield. 75%. m.p:272-274°C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm):2.28 (s,3H,-CH_3), 6.87 (s,1H,=CH), 6.98 (d,2H, *J*=4.1Hz, Ar-H), 7.10 (d,2H, *J*=4.1Hz, Ar-H), 7.49 (t, 1H, *J*=3.6Hz, benz-H), 7.54 (t, *J*=3.9Hz, 1H, benz-H), 7.61 (d, 1H, *J*=5.3Hz, benz-H), 7. 69 (d, 1H, *J*=5.5Hz, benz-H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 156.2, 154.1, 147.3, 144.0, 139.5, 134.2, 133.1, 132.4, 130.1, 129.3, 127.5, 125.1, 123.4, 115.2, 114.1, 22.3; MS: *m*/*z* 305 (M+H). Anal.Cacld for $C_{18}H_{12}N_2O_3$: C, 71.05; H, 3.97; N, 9.21. Found: C, 71.03; H, 3.96; N, 9.21 %.

3-(2,4-dimethylbenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4(3*H*)-dione(4c): Color: Brown white solid, Yield.78%. m.p:284-286°C; ^{1}H NMR (400 MHz, DMSO-d6, δ ppm): 2.24 (s,3H,-CH_3), 2.41 (s,3H,-CH_3), 6.75 (s, 1H,=CH), 6.89 (d,1H, *J*=4.2Hz, Ar-H), 7.04 (s,1H,Ar-H), 7.13 (d,1H,*J*=4.2Hz, Ar-H), 7.39 (t,1H, *J*=3.7Hz, benz-H), 7.49 (t, 1H, *J*=3.9Hz, benz-H),7.58 (d, 1H, *J*=4.9Hz, benz-H), 7. 72 (d, 1H, *J*=5.2Hz, benz-H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 156.4, 154.2, 147.3, 144.0, 138.3, 136.2, 135.1, 134.3, 132.7, 131.1, 130.1, 128.5, 127.4, 125.1, 123.6, 122.1, 117.1, 20.5, 19.5; MS: *m*/*z* 319 (M+H). Anal.Cacld for $C_{19}H_{14}N_2O_3$: C, 71.69; H, 4.43; N, 8.80. Found: C, 71.68; H, 4.42; N, 8.78 %.

3-(4-nitrobenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4] oxazine-1,4(3*H*)-dione(4d): Color: Brown solid, Yield. 58%. m.p:257-259°C; ^{1}H NMR (400 MHz, DMSO-d6, δ ppm): 6.98 (s, 1H,=CH), 7.48 (d,2H, *J*=4.6Hz, Ar-H), 7.52 (d,2H, *J*=4.8Hz, Ar-H), 7. 74 (t, 1H, *J*=5.4Hz, benz-H), 7.79 (t, 1H, *J*=5.6Hz, benz-H), 7. 84 (d, 1H, *J*=6.2Hz, benz-H), 7. 89 (d, 1H, *J*=6.5Hz, benz-H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 159.3, 156.1, 149.0, 146.1, 144.0, 142.2, 136.3, 134.5, 131.1, 129.3, 127.2, 125.1, 123.4, 119.2, 117.5; MS: *m*/*z* 336 (M+H). Anal.Cacld for $C_{17}H_9N_3O_5$: C, 60.90; H, 2.71; N, 12.53. Found: C, 60.88; H, 2.70; N, 12.51 %.

3-(4-chlorobenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*] [1,4]oxazine-1,4(3*H*)-dione(4e): Color: Light White solid, Yield, 61%. m.p:238-240°C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.95 (s, 1H, =CH), 7.56 (d, 2H, *J*=6.2Hz, Ar-H), 7.65 (d, 2H, *J*=6.5Hz, Ar-H), 7. 82 (t, 1H, *J*=5.6 Hz, benz-H), 7.87 (t, 1H, *J*=5.8 Hz, benz-H), 7. 95 (d, 1H, *J*=7.2 Hz, *J*= benz-H), 8. 04 (d, 1H, *J*=7.4 Hz, benz-H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 159.3, 156.1, 147.4, 145.0, 137.2, 135.4, 134.2, 132.0, 131.2, 130.2, 128.2, 125.1, 122.4, 118.3, 116.7; MS: *m*/*z* 325 (M+H), 327 (M+2). Anal.Cacld for $C_{17}H_9ClN_2O_3$: C, 62.88; H, 2.79; N, 8.63. Found: C, 62.87; H, 2.78; N, 8.61 %.

3-(3,5-dichlorobenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4(3*H*)-dione (4f): Color: White color solid, Yield, 57%. m.p:249-251°C;^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.98 (s, 1H, =CH), 7.66 (s,1H,Ar-H), 7.70 (s,1H,Ar-H), 7.75 (s,1H,Ar-H), 7.82 (t,1H, *J*=6.4Hz,benz-H), 7.95 (t,1H, *J*=6.8Hz,benz-H), 8.10 (d,1H, *J*=7.5Hz,benz-H), 8.15 (d,1H, *J*=7.7Hz, benz-H). MS: *m*/*z* 359 (M+H), 361 (M+2); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 161.3, 156.1, 148.4, 145.0, 137.3, 136.2, 135.2, 134.5, 132.5, 129.8, 127.2, 125.1, 123.4, 119.2, 117.1; Anal. Cacld for $C_{17}H_8Cl_2N_2O_3$: C, 56.85; H, 2.25; N, 7.80. Found: C, 56.84; H, 2.24; N, 7.89 %.

3-(4-bromobenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*] [1, 4]oxazine-1,4(3*H*)-dione(4g): Color: Light Yellow solid, Yield, 60%.m.p:239-241°C;^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.98 (s, 1H, =CH), 7.56 (d,2H, *J*=6.2Hz,Ar-H), 7.63 (d,2H, *J*=6.4Hz,Ar-H), 7.79 (t,1H, *J*=5.1Hz,benz-H), 7.86 (t,1H, *J*=5.3Hz, benz-H), 7.92 (d, 1H, *J*=6.5Hz, *J*=benz-H), 7.98 (d, 1H, *J*=6.7Hz, benz-H);^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 160.2, 156.1, 147.4, 145.0, 136.2, 134.3, 133.2, 132.5, 130.2, 127.2, 126.1, 124.3, 122.4, 117.5, 115.3; MS: *m*/*z* 369 (M+H), 371 (M+2); Anal.Cacld for $C_{17}H_9BrN_2O_3$: C, 55.31; H, 2.46; N, 7.59. Found: C, 55.30; H, 2.45; N, 7.58 %.

3-(4-methoxybenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*] [1,4]oxazine-1,4(3*H*)-dione(4h): Color: Light Brown solid, Yield, 72%. m. p: 247-249°C; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 3.82 (s, 3H, -OCH_3), 6.79 (s, 1H, =CH), 7.39 (d,2H, *J*=5.1Hz, Ar-H),7.49 (d,2H, *J*=5.4Hz, Ar-H), 7.65 (t,1H, *J*=4.6Hz, benz-H), 7.71 (t,1H, *J*=4.8Hz, benz-H), 7.82 (d, 1H, *J*=6.5Hz, benz-H), 7.90 (d, 1H, *J*=6.3Hz, benz-

H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 160.1, 159.1, 155.2, 147.4, 144.2, 136.2, 134.3, 132.7, 131.7, 127.2, 124.2, 121.3, 119.4, 116.6, 114.3, 56.2; MS: *m/z* 321 (M+H); Anal.Cacld for $C_{18}H_{12}N_2O_4$: C, 67.50; H, 3.78; N, 8.75. Found: C, 67.48; H, 3.77; N, 7.58 %.

3-(3,5-dimethoxybenzylidene)-1*H*-benzo[4,5]imidazo [2, 1-*c*][1,4]oxazine-1,4-(3*H*)-dione (4i): Color: Light white solid, Yield, 72%. m. p: 271-273ºC; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 3.92 (s, 3H, -OCH_3), 4.00 (s, 3H, -OCH_3), 6.90 (s, 1H, =CH), 7.25 (s,1H, Ar-H), 7.34 (s,1H, Ar-H), 7.42 (s,1H, Ar-H), 7.49 (t,1H, *J*=4.3Hz, benz-H), 7.62 (t,1H, *J*=4.5Hz, benz-H), 7.68 (d, 1H, *J*=5.2Hz, benz-H), 7.75 (d, 1H, *J*=5.4Hz,benz-H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm):163.9, 163.1, 159.3, 156.0, 147.3, 145.0, 139.5, 136.4, 134.3, 128.5, 126.2, 124.3, 119.3, 117.1, 109.2, 58.6; MS: *m/z* 351 (M+H).Anal.Cacld for $C_{19}H_{14}N_2O_5$: C, 65.14; H, 4.03; N, 8.00. Found: C, 65.13; H, 4.00; N, 8.00 %.

3-(3,4-dichlorobenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4-(3*H*)-dione (4j): Color: Light White solid. Yield,51%. m. p: 261-263ºC; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 6.98 (s, 1H, =CH), 7.50 (d,1H, *J*=6.5Hz, Ar-H), 7.55 (d,1H, *J*=6.7, Hz,Ar-H), 7.60 (s,1H, Ar-H), 7.74 (t,1H, *J*=5.4Hz, benz-H), 7.76 (t,1H, *J*=5.6Hz,benz-H), 7.80 (d, 1H, *J*=6.3Hz, benz-H), 7.86 (d, 1H, *J*=6.7Hz, benz-H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 159.2, 156.2, 148.4, 145.0, 136.3, 134.6, 133.0, 132.6, 131.3, 130.1, 129.4, 127.1, 127.7, 125.4, 123.4, 119.3, 116.4; MS: *m/z* 358 (M+H), 360 (M+2): Anal. Cacld for $C_{17}H_8Cl_2N_2O_3$: C, 56.85; H, 2.25; N, 7.80. Found: C, 56.83; H, 2.24; N, 7.78 %.

4-((1,4-dioxo-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-3 (4*H*)-ylidene)methyl)benzo nitrile (4k): Color: White Brown solid, Yield, 51%. m. p: 252-254ºC; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 6.84 (s, 1H, =CH), 7.61 (d, 2H, *J*=6.2Hz, Ar-H), 7.66 (d,2H, *J*=6.3Hz, Ar-H), 7.83 (t,1H, *J*=5.1Hz, benz-H), 7.87 (t,1H, *J*=5.3Hz, benz-H), 7.95 (d, 1H, *J*=6.2Hz, benz-H), 8.06 (d, 1H, *J*=6.5Hz, benz-H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 159.3, 156.1, 147.3, 144.0, 139.2, 138.5, 136.4, 134.2, 133.2, 127.2, 125.1, 124.4, 120.1, 119.5, 117.1, 113.7; MS: *m/z* 316 (M+H): Anal.Cacld for $C_{18}H_9N_3O_3$: C, 68.57; H, 2.88; N, 13.33. Found: C, 68.55; H, 2.87; N, 13.32 %.

3-(4-fluorobenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*] [1, 4]oxazine-1,4-(3*H*)-dione(4l): Color: Light brown solid, Yield, 54 %. m. p: 257-259ºC; ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 6.92 (s, 1H, =CH), 7.64 (d,1H, *J*=5.3Hz, Ar-H), 7.69 (d,1H, *J*=5.6Hz, Ar-H), 7.91 (t,1H, *J*=5.4Hz, benz-H), 7.97 (t,1H, *J*=5.6Hz, benz-H), 8.05 (d, 1H, *J*=6.2Hz,benz-H), 8.12 (d, 1H, *J*=6.5Hz, benz-H); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 164.3, 160.4, 157.1, 148.4, 144.3, 136.5, 134.5, 133.1, 132.3, 129.2, 127.3, 124.5, 120.2, 118.1, 116.7; MS: *m/z* 309 (M+H): Anal.Cacld for $C_{17}H_9FN_2O_3$: C, 66.24; H, 2.94; N, 9.09. Found: C, 66.22; H, 2.93; N, 9.06 %.

Antimicrobial activity: Antimicrobial activities of compounds **(**4a-l) were carried out by broth dilution method against test organisms. Using the sterile cork borer, wells 6 mm were made into each Petri plate. DMSO was used as a negative control. The test compounds and standard drugs are dissolved in DMSO of specific concentrations 200 and 400 µg/ mL; test compounds are filled in the wells and incubated at 37^0C and the diameter of the inhibition zones was measured after 24 hours in case of bacteria and after 48 hours in case of fungi. After appropriate incubation, the diameter of the zone of inhibition of each well was measured. Ciprofloxacin was used as a reference drug for antibacterial activity agents. Itrazole drug was used as a reference for an anti-fungal agent. DMSO was used as a negative control. Duplicates were maintained and the average values were calculated for eventually antibacterial and antifungal activity.

DPPH free radical scavenging assay: The synthesized compounds (**4a-l)** were screened for *in vitro* antioxidant activity in terms of hydrogen donating or radical scavenging ability by rapid and convenient technique i.e. 1,1-diphenyl-2-picrylhydrazyl (DPPH) Assay using ascorbic acid as standard drugs. Methanol (95%), DPPH solution and standard drugs were used as a blank, control and reference respectively. Absorbance was calculated at 517 nm (at an absorption maximum of DPPH) after keeping the mixture of 100 mL of synthesized compounds of concentration 10 µg mL^{-1} (dissolved in DMSO) and 900 mL of DPPH radical solution (0.004% w/v of DPPH in methanol) in a dark place for 30 min incubation period.

Antioxidant activity was expressed in terms of IC_{50} (µM), the effective concentration at which 50% of the radicals were scavenged. Evaluation of the antioxidant activity revealed that the tested compounds exhibited good to excellent DPPH radical scavenging ability.

Results and Discussion

The synthetic approach of targeted compounds was illustrated in scheme I. Initially *O*-phenylenediamine (1) was treated with glyoxalic acid in 4N HCl at 90ºC for 4 hours to give (1*H*-benzo[d]imidazol-2-yl) methanol (2). Later the compound (2) was oxidized with $KMnO_4$ in 20% NaOH at reflux temperature for 1 hr to give 1*H*-Benzo[d]imidazole-2-carboxylic acid[20]. The intermediate was treated with chloro acetyl chloride in dry THF and add Cs_2CO_3 as a base. The mixture was stirred at 80ºC for 5-6 h in formation of 1*H*-benzo[4,5]imidazo[2,1-*c*]oxazine-1,4-(3*H*)-dione (3).

Finally, the compound (3) was condensed with different substituted aromatic aldehydes in ethanol by adding catalytic amount of *p*-toluene sulphonyl chloride (*p*-TsCl) providing the targeted compounds (4a-l) in moderate to high yields.

The structures of the newly synthesized compounds (4a-l) were analyzed by ^{1}H NMR spectroscopy, ^{13}C NMR, ESI-Mass spectrometry and CHN analysis techniques. For a representative compound (4a) in the IR spectrometer, the intense band at 3040 cm^{-1} is due to aromatic C-H stretching, the band at 1756 cm^{-1} is attributed to formation of amide and sharp bands at 1623 and 1525 cm^{-1} due to –N=N and –C=N stretching vibrations respectively. In the ^{1}H NMR, the presence of singlet signal at δ 6.98 ppm supports the presence of benzaldehyde proton. Similarly, in the ^{13}CNMR spectrum, a peak appears at δ 134.6 ppm confirming the presence of olefinic carbon atom attached to oxazine-1,4-(3*H*)-dione. The mass spectrum of the compound (4a) showed $[M+H]^{+}$ peak at m/z 291 (M+H).

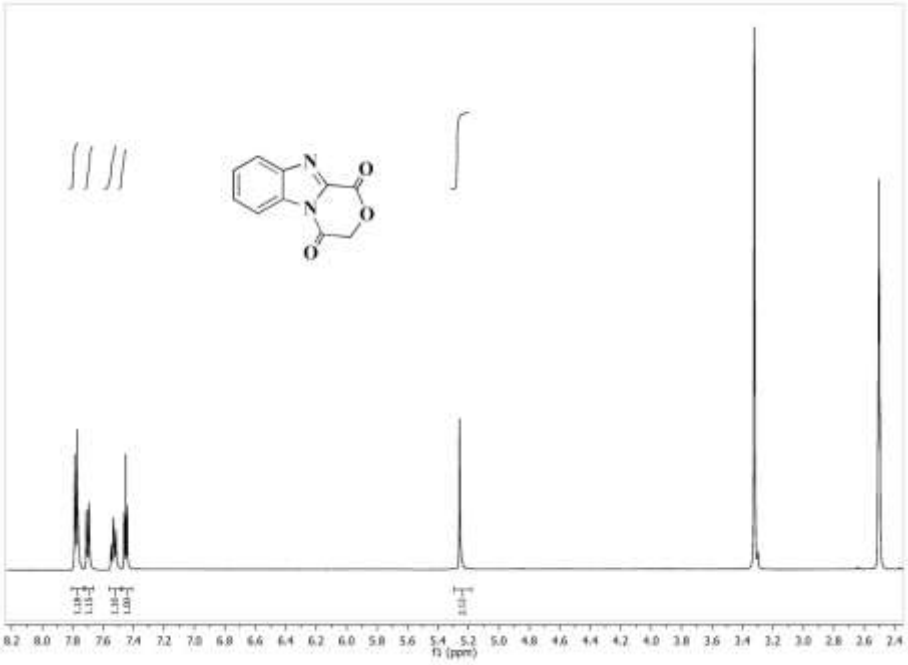


Figure 1: NMR spectra of 1*H*-benzo[4,5]imidazo[2,1-*c*]oxazine-1,4-(3*H*)-dione

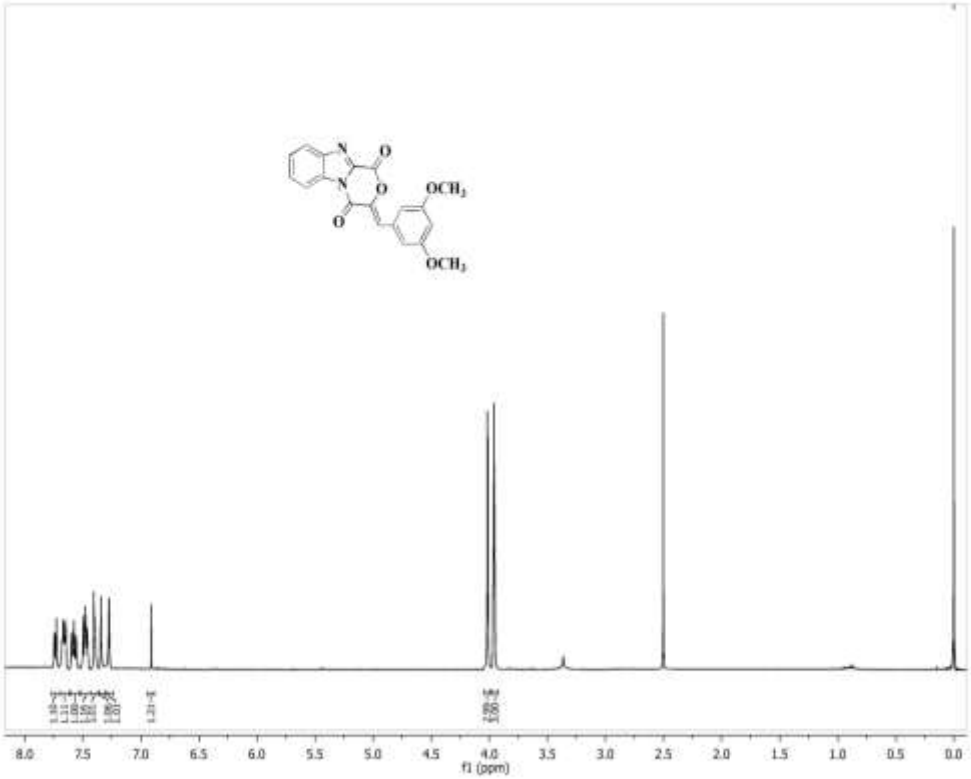


Figure 2: ^{1}HNMR spectra of 3-(3,5-dimethoxybenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4-(3*H*)-dione

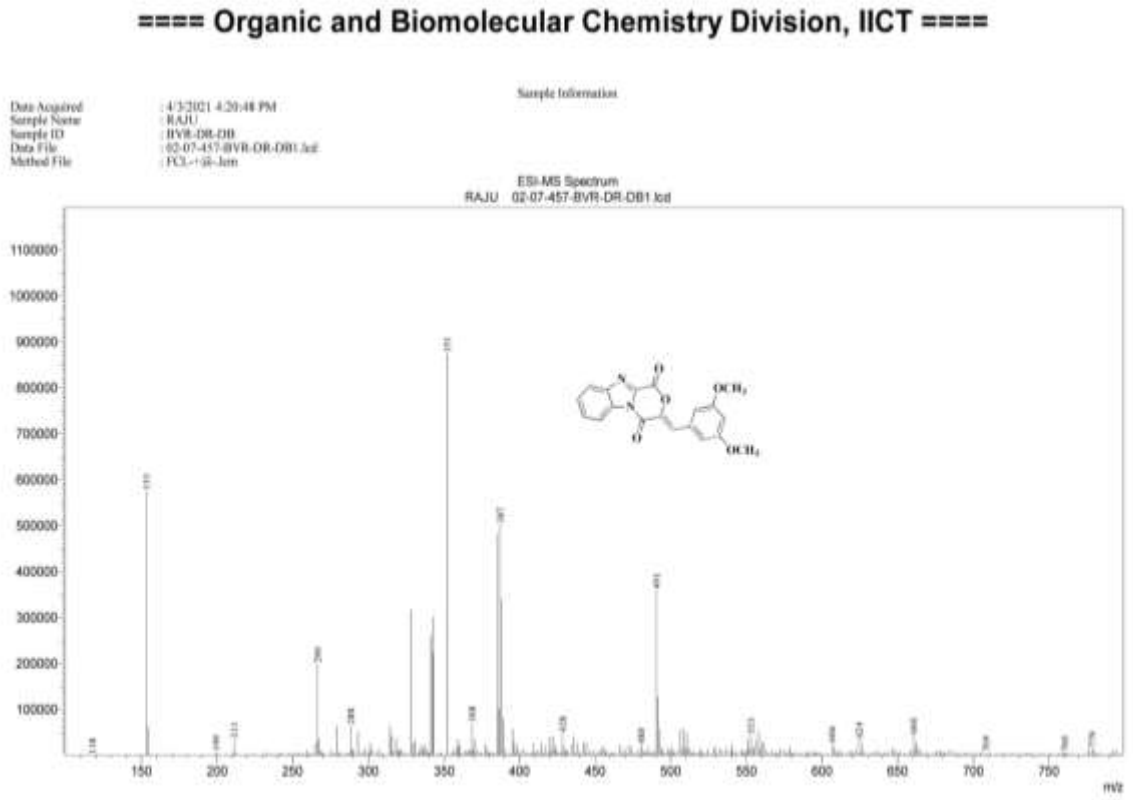


Figure 3: Mass spectra of 3-(3,5-dimethoxybenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4-(3*H*)-dione

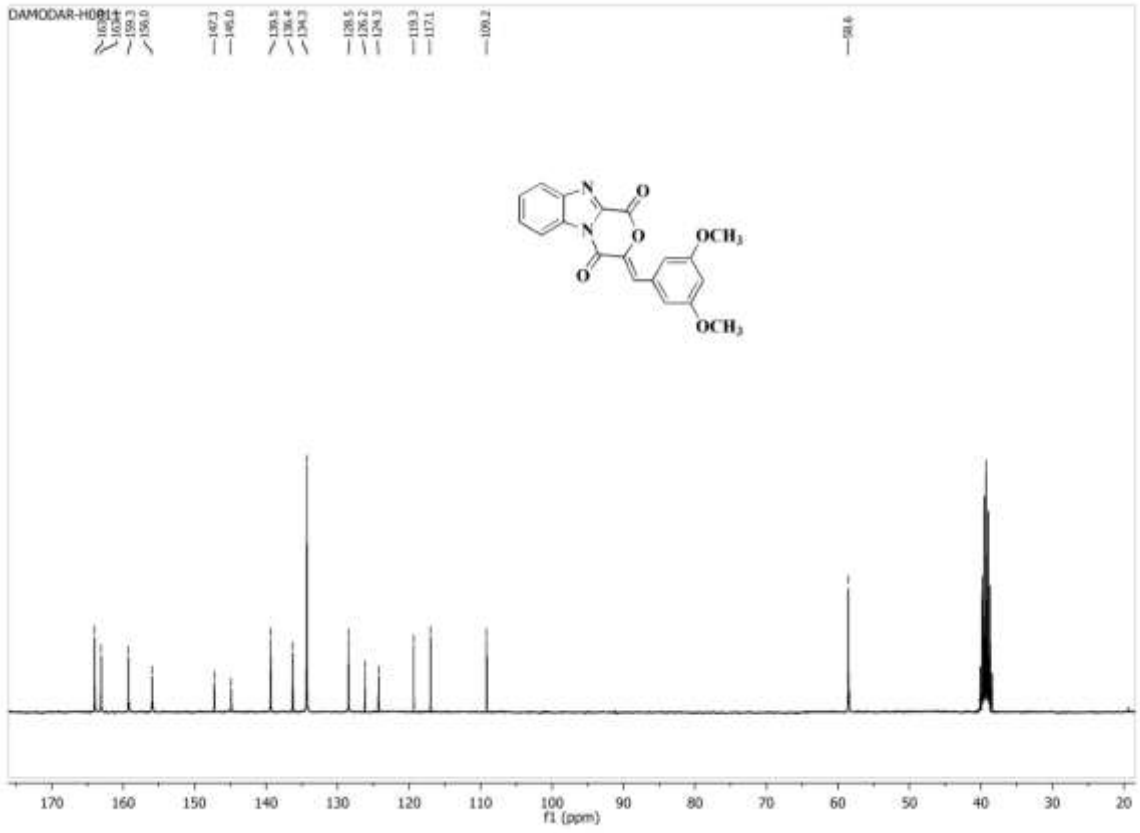


Figure 4: ^{13}CNMR spectra of 3-(3,5-dimethoxybenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine -1,4-(3*H*)-dione(4i).

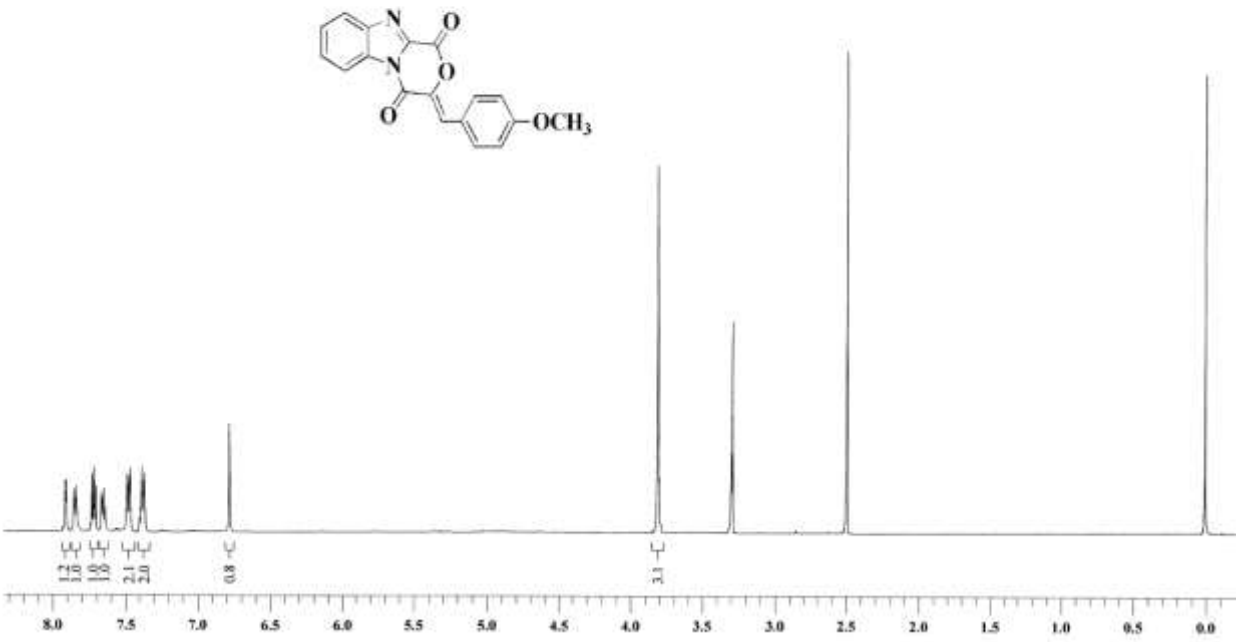


Figure 5: [1]HNMR Spectra of 3-(4-methoxybenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4(3*H*)-dione

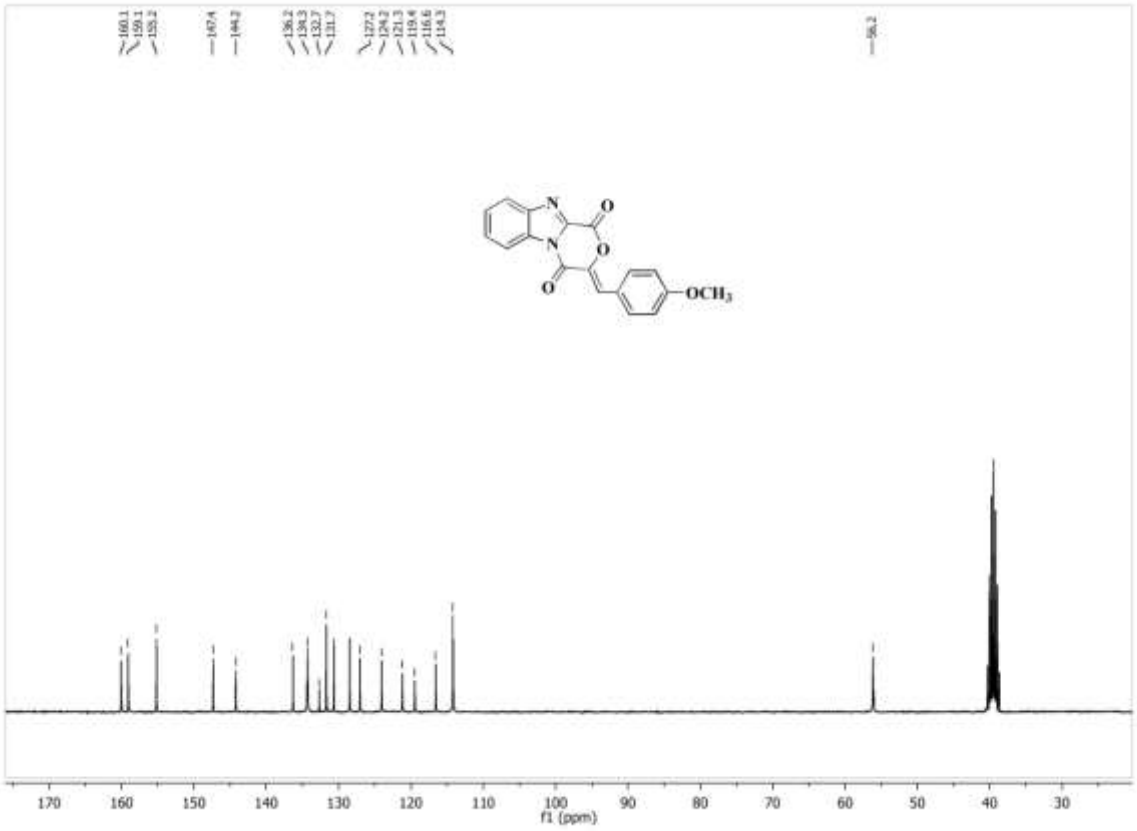


Figure 6: [13]CNMR spectra of 3-(4-methoxybenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4(3*H*)-dione

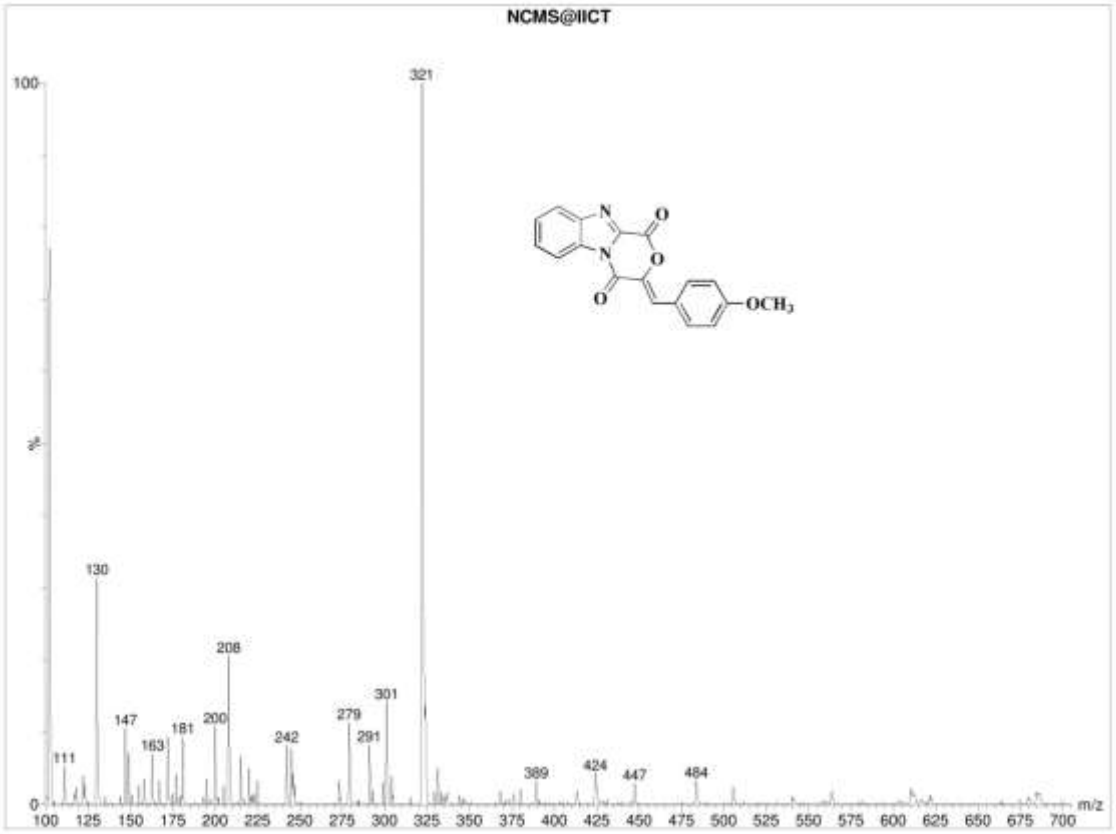


Figure 7: Mass spectra of 3-(4-methoxybenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4(3*H*)-dione

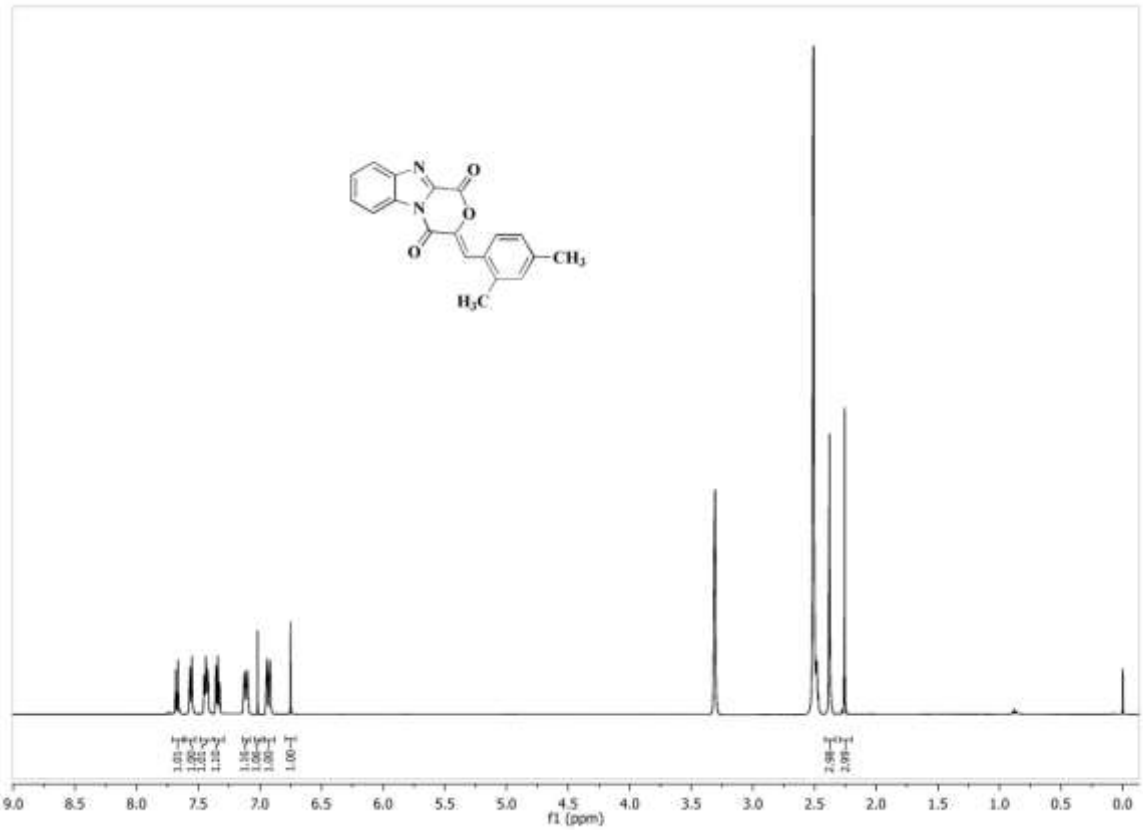


Figure 8: ^{1}HNMR spectra of 3-(2,4-dimethylbenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4(3*H*)-dione

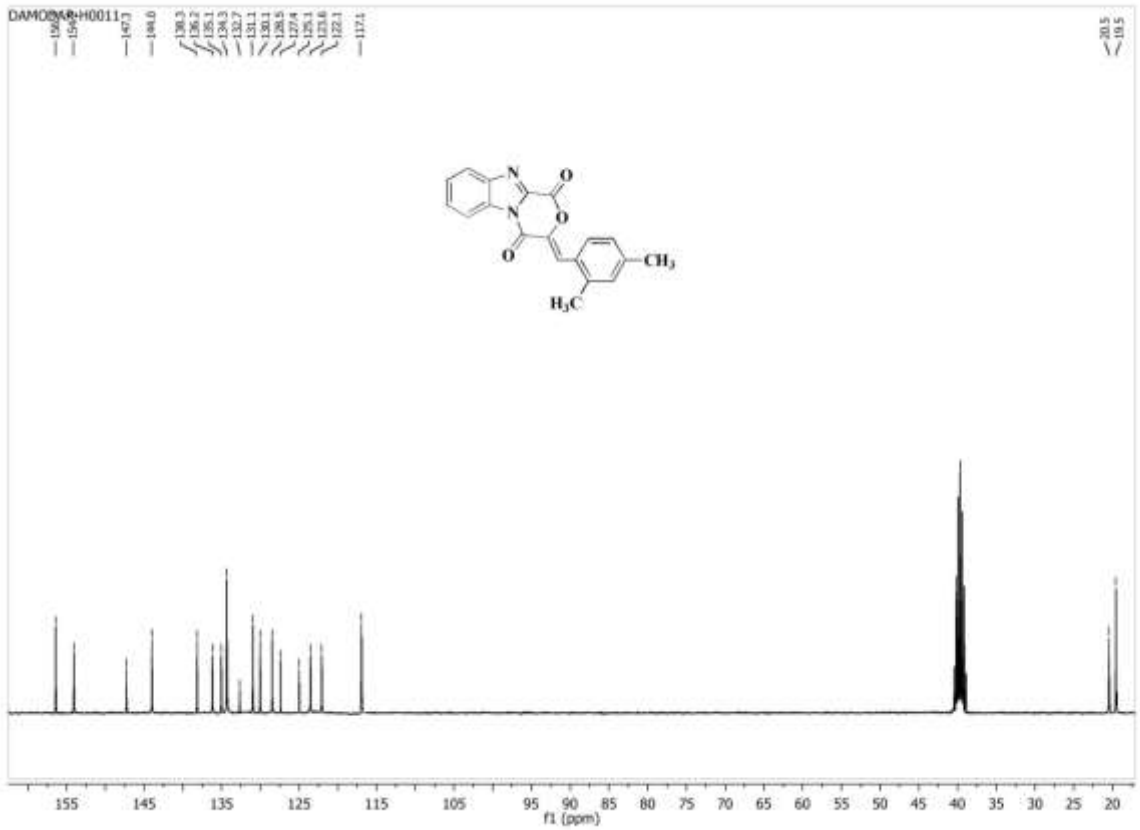


Figure 9: ^{13}CNMR spectra of 3-(2,4-dimethylbenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4(3*H*)-dione

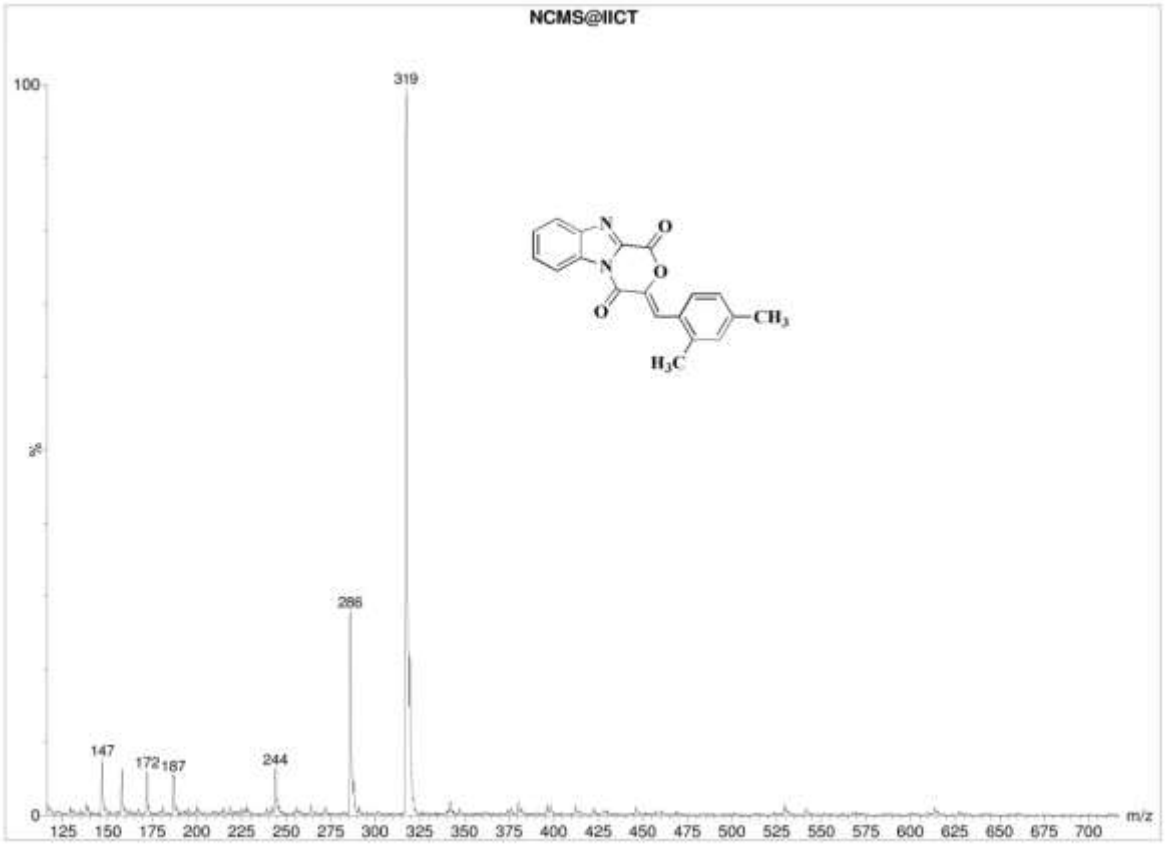


Figure 10: Mass spectra of 3-(2,4-dimethylbenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxazine-1,4(3*H*)-dione

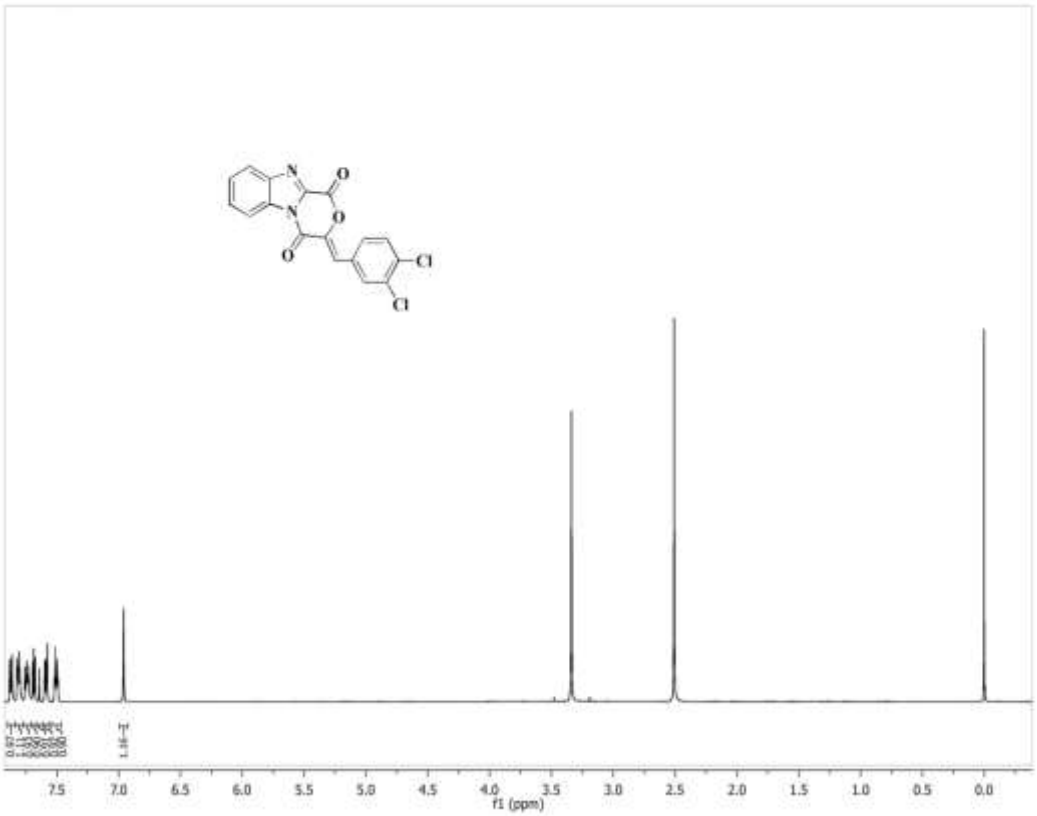


Figure 11: [1]HNMR spectra of 3-(3,4-dichlorobenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4]oxaz ine-1,4-(3*H*)-dione

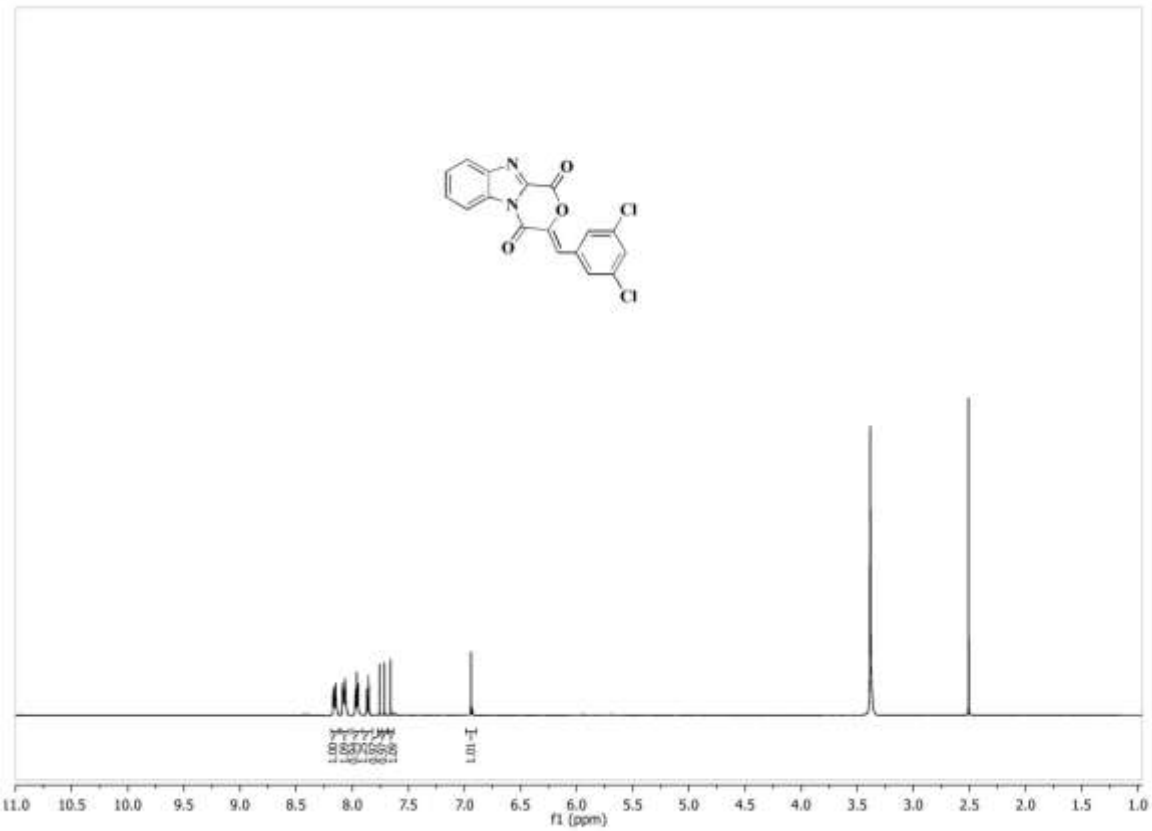


Figure 12: [1]HNMR spectra of 3-(3,5-dichlorobenzylidene)-1*H*-benzo[4,5]imidazo[2,1-*c*][1,4] oxaz ine-1,4(3*H*)-dione

Conclusion

We reported a simple and efficient method for the synthesis of novel 3-benzylidene-1*H*-benzo [4,5]imidazo[2,1-*c*][1,4]oxazine-1,4(3*H*)-dione derivatives (4a-l) in moderate to good yields. The synthesized compounds were screened for their anti-microbial and anti-oxidant activity. The activity results reveal that the compounds (4i) showed prominent activity and remaining (4h), (4f) and (4j) showed good activity against all the tested bacterial strains compared to standard streptomycin drug.

The anti-fungal activity results also showed that the compounds (4i), (4h) and (4f) exhibited potent activity against *Candida albucance, Fusariumoxysporium* compared to Itrazole drug. The anti-oxidant activity studies revealed that the compound (4i) displayed promising activity whereas the compounds (4f) and (4h) showed potent activity as compared to the other tested compounds.

Acknowledgement

The authors are thankful to the Head of Department of Chemistry for providing a research facility at Kakatiya University. The authors are also thankful to the Director of the Indian Institute of Chemical Technology and the Director of Center for Cellular and Molecular Biology Hyderabad for providing spectral data and biological activity.

References

1. Ansari K. and Lal C., Synthesis and evaluation of some new benzimidazole derivatives as potential antimicrobial agents, *European Journal of Medicinal Chemistry*, **44(5)**, 2294-2299 **(2009)**

2. Anjana D.T., Jain N., Kalivendi S.V., Murthy R., Patpi S.R., Pulipati L., Sridhar B., Sriram D. and Yogeeswari P., Design, synthesis and structure-activity correlations of novel dibenzo[b,d]furan, dibenzo[b,d]thiophene and N-methylcarbazole Clubbed 1,2,3-Triazoles as Potent Inhibitors of Mycobacterium tuberculosis, *J Med Chem*, **55(8)**, 3911-3922 **(2012)**

3. Aurelio S., Ines Tapia, Luisa Alonso-Cires, Luis Labeaga, Pedro Lopez-Tudanca and Ramon Mosquera, Benzimidazole-2-carboxylic acid amides and esters: a new structural class of 5-HT3 ligands, *European Journal of Medicinal Chemistry*, **34(12)**, 415-422 **(1999)**

4. Bastide J.M., Blancard A. and Mallie M., *In vitro* susceptibility testing of candida and aspergillus spp. to voriconazole and other antifungal agents using Etest: results of a French multicentre study, *International Journal of Antimicrobial Agents*, **25(4)**, 321-328 **(2005)**

5. Bai A.L., Tang J.M. and Zhang J.J., Synthesis and Antifungal Activity of 2-Chloromethyl-1*H*-benzimidazole Derivatives against Phytopathogenic Fungi *in Vitro*, *Journal of Agricultural and Food Chemistry*, **61**, 2795-2789 **(2013)**

6. Bagul C., Kamal A., Rao M.P.N., Shaik A.B., Srinivasulu V., Swapna P., Kovvuri J., Mullagiri K., Nagesh N., Reddy V.S. and Vidyasagar K., Synthesis of β-carboline–benzimidazole conjugates using lanthanum nitrate as a catalyst and their biological evaluation, *Organic & Biomolecular Chemistry*, **12**, 2370-2387 **(2014)**

7. Buckle D.R., Outred D.J., Rockell C.J.M. and Smith H.B., Studies on 1, 2, 3,-triazoles. 10. Synthesis and antiallergic properties of 9-oxo-1H, 9H-benzothiopyrano [2, 3-d]-1, 2, 3-triazoles and their S-oxides, *J Med Chem*, **27(2)**, 223-227 **(1984)**

8. Brik A., Cauvi G., Elder J., Giffin M.J., Heaslet H., Lin Y.C., Mc Ree D.E. and Wong C.H., A copper(I)-catalyzed 1,2,3-triazole azide-alkyne click compound is a potent inhibitor of a multidrug-resistant HIV-1 protease variant, *J Med Chem*, **51(20)**, 6263-6270 **(2008)**

9. Boechat N., Castro H.C., Costa M.S., Junior I.N., Rangel E., Rodrigues C.R., Silva A. and Souza M.T.D., Synthesis, tuberculosis inhibitory activity and SAR study of N-substituted-phenyl-1,2,3-triazole derivatives, *Bioorganic & Medicinal Chemistry*, **14(24)**, 8644-8653 **(2006)**

10. Shireen Mohammed, Alya Dawood and Mohammed Mohammed, Synthesis, Identification and Biological Activity of some Schiff Bases derived from 1,5-diamino naphthalene substrate, *Res. J. Chem. Environ.*, **23(9)**, 108-112 **(2019)**

11. Battula K.S., Narsimha S. and Reddy N.V., One-pot synthesis of novel 1,2,3-triazole-pyrimido[4,5-c]isoquinoline hybrids and evaluation of their antioxidant activity, synthetic communications, *Synthetic Communications*, **48(10)**, 1220-1226 **(2018)**

12. Cai G.X., Peng X.M. and Zhou C.H., Recent Developments in Azole Compounds as Antibacterial and Antifungal Agents, *Current Topics in Medicinal Chemistry*, **13(16)**, 1963-2010 **(2013)**

13. Chandrakant B., Kamal A., Mullagiri K., Nayak V.L., Ponnampalli S., Rao M.P.N. and Vishnuvardhan M.V.P.S., Synthesis of imidazothiadiazole–benzimidazole conjugates as mitochondrial apoptosis inducers, *Med Chem Comm*, **5**, 1644-1650 **(2014)**

14. Deepthi S.B. et al, Carbohydrate triazole tethered 2-pyridyl-benzimidazole ligands: Synthesis of their palladium (II) complexes and antimicrobial activities, *Inorganica Chimica Acta*, **435**, 2000-2005 **(2015)**

15. Demaray J.A., Dawson M.N., Sucheck S.J. and Thuener J.E., Synthesis of triazole-oxazolidinones via a one-pot reaction and evaluation of their antimicrobial activity, *Bioorganic & Medicinal Chemistry Letters*, **18(17)**, 4868-4871 **(2008)**

16. Elder J.H., Lin Y.C., Lindstrom W., Olson A.J., Sharpless K.B., Tripp J.C. and Whiting M., Rapid discovery and structure-activity profiling of novel inhibitors of human immuno deficiency virus type 1 protease enabled by the copper(I)-catalyzed synthesis of 1,2,3-Triazoles and their further functionalization, *J Med Chem*, **49(26)**, 7697-7710 **(2006)**

17. He Y., Risen L., Swayze E.E., Wu B., Yang J., Wu B. and Swayze E.E., Synthesis and biological evaluations of novel benzimidazoles as potential antibacterial agents, *Bioorganic & Medicinal Chemistry Letters*, **14(5)**, 1217-1220 **(2004)**

18. Hu G., Liu J., Xu X., Yan Z. and Zhang R., A reverse method for diversity introduction of benzimidazole to synthesize H^+/K^+-

ATP enzyme inhibitors, *Bioorganic & Medicinal Chemistry Letters*, **21(14)**, 4189-4192 **(2011)**

19. Hundal G., Gupta R., Kaushik N.K., Singh A.P. and Verma A.K., Synthesis, structure and biological activity of copper(II) complexes of 4-(2-pyridylmethyl)-1,7-dimethyl-1,4,7-triazonane-2,6-dione and 4-(2-pyridylethyl)-1,7-dimethyl-1,4,7-triazonane-2,6-dione, *European Journal of Medicinal Chemistry*, **44(4**), 1607-1614 **(2009)**

20. Kaustovaa J., Klimessovaa V., Kocci J., Pour M., Stachel J. and Waisser K., Synthesis and preliminary evaluation of benzimidazole derivatives as antimicrobial agents, *European Journal of Medicinal Chemistry*, **44(10)**, 4028-4033 **(2009)**

21. Luxami V., Paul K. and Singla P., Benzimidazole-biologically attractive scaffold for protein kinase inhibitors, *RSC Advances*, **4**, 12422-12440 **(2014)**

22. Narsimha S., Ramesh B. and Ravinder M., Synthesis and Biological Evaluation of New 1,2,3-Triazole Based 2-Sulfonylbenzoxazoles as Potent Anti-inflammatory and Anti bacterial Agents, *Indian Journal of Heterocyclic Chemistry*, **29**, 389-395 **(2019)**

23. Wan K., Wang X.L. and Zhou C.H., Synthesis of novel sulfanilamide-derived 1,2,3-triazoles and their evaluation for antibacterial and antifungal activities, *European Journal of Medicinal Chemistry*, **45(10)**, 4631-4639 **(2010)**.

(Received 02nd April 2021, accepted 09th June 2021)